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Reproductive Morphology of Acerola (*Malpighia glabra* L.)

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Reproductive Morphology of Acerola (*Malpighia glabra* L.)[1]

R. K. MIYASHITA, H. Y. NAKASONE, and C. H. LAMOUREUX

INTRODUCTION

Studies to date *(16, 17)* have shown that fruit set of acerola grown in Hawaii was extremely low despite prolific flowering and that it was primarily due to the absence of pollinating agents. Fruit set was increased by hand pollination and by growth regulator application. It was reported that seed production was very low with many clones producing almost no viable seeds. Also, flowers emasculated and bagged to prevent pollination set a few fruits, all lacking viable seeds.

The highly responsive nature of the flowers to growth regulators, the low order of viable embryo production despite hand pollination, and the successful setting of a few emasculated flowers without pollination indicated presence of parthenocarpy of some degree.

Problems concerning pollination, fruit set, and ovule sterility are important considerations in any breeding program. The low seed production in acerola has been the major deterrent to breeding of specific types. Further studies appeared desirable, especially in the area of reproductive morphology. This paper reports the results of such studies. Associated problems involving floral bud differentiation, pollen development, and pollen germination were investigated and are reported herein.

BOTANICAL DESCRIPTION

Detailed descriptions of this plant have been given adequately by several workers *(1, 2, 6, 17)* and therefore it seems necessary only to present a brief description of the major characteristics. Botanically, the acerola, or West Indian cherry, is known as *Malpighia glabra,* belonging to the family Malpighiaceae. It is native to Tropical America and first gained prominence in 1946 when unusually high amounts of ascorbic acid were found in the fruit *(2).*

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[1] This technical bulletin is part of a thesis submitted by the senior author to the Graduate School of the University of Hawaii in partial fulfillment of the requirements for the Master of Science degree.

The plant is a small tree or a shrub with upright or drooping habit, attaining heights of 3 to 4 meters. The flowers (fig. 1*a*) are 2.0 to 2.5 centimeters in diameter, borne on simple dichasium inflorescences in the leaf axils. There are five green sepals, five pink petals, ten stamens, and three carpels fused into a superior ovary.

In Hawaii the flowering cycles occur approximately every 25 days, commencing in early spring and continuing into late fall and even into winter. The fruit (fig. 1*b*, 62) is a trilocular drupe with shallow lobes and thin skin. It is light orange to dark red in color when ripe and weighs from 2 to 10 grams. It has a juicy mesocarp.

REVIEW OF LITERATURE

Investigations on gametogenesis and embryogeny of the Malpighiaceae have not been extensive. According to Narasimhachar *(8)* and Rao *(10)*, Braun in 1860 reported polyembryony in *Banisteria* and *Stigmatophyllum* as the first of such investigations for this family. Ritzerow *(11)* mentioned nucellar polyembryony in three species of Aspicarpa and the lack of fertilization due to abnormal pollen. All embryos observed were situated at the chalazal end of the ovule. Rao *(10)* reported that fertilization also did not occur in *Banisteria laurifolia, Stigmatophyllum aristatum,* and *Hiptage madablota* because of abnormal pollen, and the poor organization and early degeneration of the embryo sacs. He also reported the occurrence of free-nuclear endosperm which resulted from division of the fusion nucleus and micropylar embryos of nucellar origin in *H. madablota,* while *B. laurifolia* and *S. aristatum* showed neither embryo nor endosperm formation.

Schurhoff *(13)* briefly described megagametogenesis in *Malpighia coccifera, M. urens,* and *Bunchosia nitida* and reported that all three species possessed 16-nucleate embryo sacs. This work was confirmed by Stenar *(15)* in *M. urens,* by Rao *(10)* in *B. laurifolia, S. aristatum,* and *H. madablota,* and by Narasimhachar *(8)* in *M. punicifolia.*[2] Rao reported that embryo sac development was like the *Penaea*-form

[2] *M. glabra* L. and *M. punicifolia* L. refer to the same species with the former binomial reported to be the first one used. To conform with the International Code of Botanical Nomenclature, the original binomial, *M. glabra* L., is used in this paper.

PLATE I

FIGURE 1*a*. Stages of flower development from emergence of bud to anthesis of three clones. (0.338×)

FIGURE 1*b*. Stages of fruit development from anthesis to full maturity of three acerola clones. (0.188×)

FIGURE 2. Lateral shoots of Clone A in active vegetative growth and producing flowers. Arrow shows flower buds at the uppermost axil of fully expanded leaves.

FIGURE 3. Numerous axillary meristems of acerola in superposed arrangement. (10×)

FIGURE 4. Vegetative axillary meristem. Leaf primordia (*l*); apical meristem (*a*). (98×)

FIGURE 5. Differentiation of floral parts: *c, p,* and *s* show sepal, petal, and stamen differentiation, respectively. (97×)

FIGURE 6. Further development of floral parts: *c, p, s,* and *b* show development of sepal, petal, stamen, and pistil, respectively. (42×)

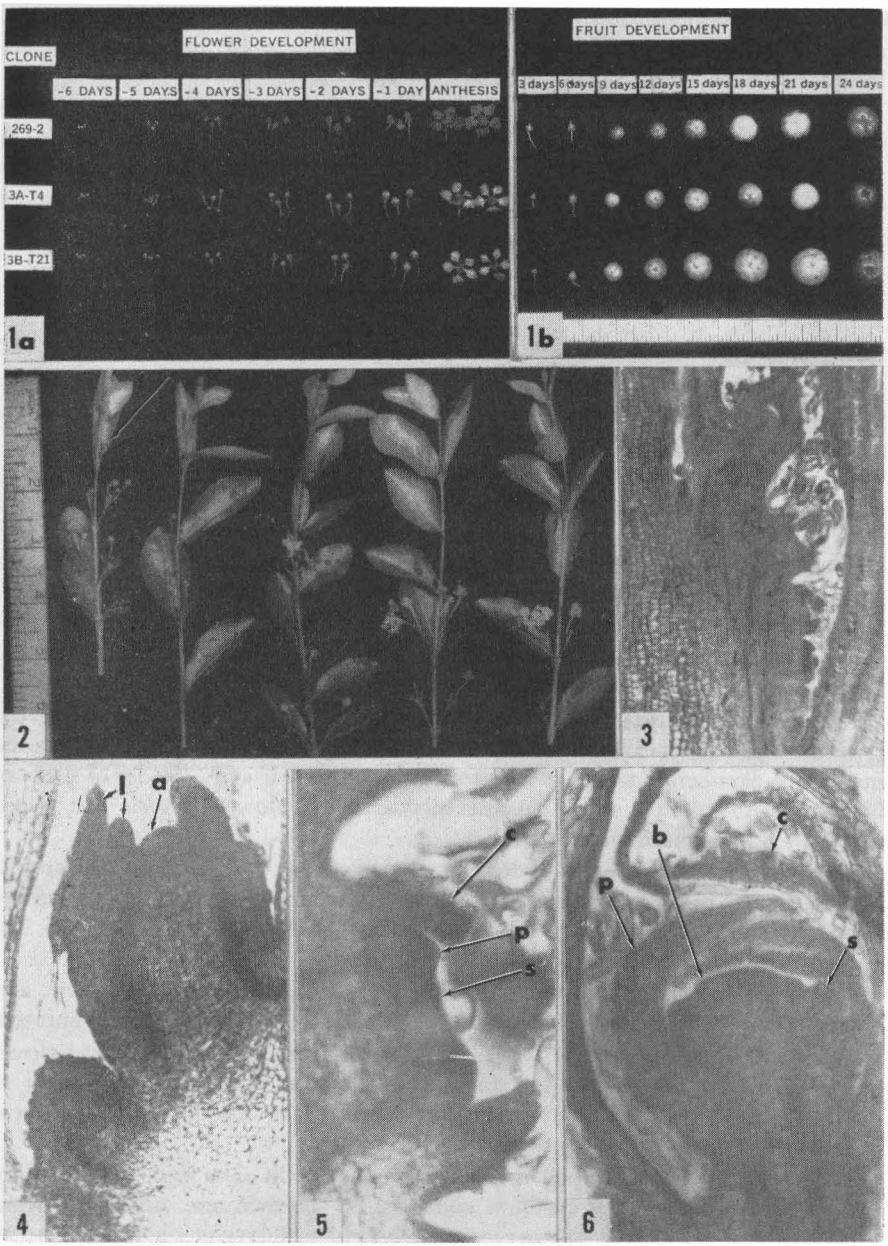
FLOWER DEVELOPMENT
CLONE
-6 DAYS
-5 DAYS
-4 DAYS
-3 DAYS
-2 DAYS
-1 DAY
ANTHESIS
269-2
3A-T4
3B-T21
1a
FRUIT DEVELOPMENT
3 days
6 days
9 days
12 days
15 days
18 days
21 days
24 days
1b
2
3
l
a
4
c
p
s
5
b
c
p
s
6

PLATE I

of the *Peperomia*-type and Narasimhachar reported a *Lilium*-type of embryo-sac development. Development of an 8-nucleate embryo sac of the *Allium*-type has been reported for *Galphimia gracilis (15)* and *M. glauca (9).*

The occurrence of several megaspore mother cells per megasporangium was reported by Rao *(10)* in *B. laurifolia, S. aristatum,* and *H. madablota* and by Narasimhachar *(8)* in *M. punicifolia.* However, in all cases, only a single megaspore mother cell was found to develop into a mature embryo sac. No polyembryony was observed in *M. punicifolia.*

MATERIALS AND METHODS

Acerola clones used in this study are identified as Nos. 269-2, 3A-T4, 3A-T8, 3B-T21, and Maunawili. Plants were approximately 3 years old. Three clones in particular, 269-2, 3A-T4, and 3B-T21, were used throughout the entire study and are referred to in this paper as clones A, B, and C, respectively.

Floral differentiation was studied by determining the growth activity of branches in active growth. For the purpose of clarity in identification of branch types, two types, (a) lateral shoots and (b) induced shoots formed on lateral branches after the tips were decapitated, were used. The lateral branches are referred to in this paper as "primary" and the induced branches as "secondary." The term "emergence" as used in this paper refers to axillary buds at the time they become distinguishable to the naked eye at a normal viewing distance (approximately 30 centimeters away from the buds).

Floral differentiation on active primary branches was studied by labelling the lowermost pair of unexpanded leaves at the tips of the branches to determine the number of days before flowers were produced in their leaf axils. Nodes of primary branches were collected at various intervals during the flowering cycle and placed in FAA *(12)* for histological studies.

Floral differentiation on secondary shoots was studied by inducing shoot growth on decapitated primary branches 20 centimeters from the apex. Growth activity of the secondary shoots and the number of days prior to emergence of the first flower buds in the leaf axils were observed daily.

Flower development from the time of emergence in the leaf axil was also observed daily to determine the number of days between emergence and anthesis.

Due to the minute size of the anthers, time of anther dehiscence was determined by rubbing the anthers of sample flowers against a black blotting paper to test for presence of pollen grains and by viewing the anthers under a stereomicroscope at intervals of approximately 30 minutes.

Pollen germination and pollen tube growth studies *in vitro* were conducted by sowing pollen on artificial medium containing 1 percent agar and 12 percent sucrose in tap water. Medium was pipetted onto micro-slides and allowed to harden. Pollen was distributed on the surface of the medium with a fine bristle brush. After sowing, the slides were incubated under high humidity (80–90 percent) at room temperature in petri dishes lined with moistened filter paper. Pollen

germination counts were made 5 hours after sowing by observing five microscopic fields through a 12.5× eyepiece and 10× objective.

Pollen tube growth through the style was studied by pollinating the stigmas and removing the styles 4 hours later for culturing on sucrose-agar medium. Styles of varying lengths (one-third, two-thirds, and entire length), all with intact stigmas, were either placed horizontally on the medium or stood upright on their cut ends to determine the period of time elapsed before the pollen tubes emerged from the cut ends.

In vivo studies of pollen germination and pollen tube growth were made from paraffin sections of flowers which were pollinated on the day of anthesis and collected daily thereafter.

Hand pollination was done by bagging branches bearing numerous flower buds just beginning to open in the morning with paper sacks. Self-pollination was accomplished the same afternoon by using a fine bristle brush to transfer the pollen. Flowers were bagged after pollination.

For cross-pollinations, anthers were emasculated before they dehisced. Emasculated flowers were kept bagged before and after pollination.

Nawaschin's Craf III *(12)* was used to kill and fix floral buds and fruits in various stages of development. Ovules from fertilized flowers were dissected from the stony endocarps before killing.

Dehydration followed the tertiary butyl alcohol method of Johansen *(5)* and was followed by infiltration with "parowax" and embedding in "Tissue-mat" (melting point 56°–58° C). Sections were cut on a rotary microtome at 12 to 15 microns.

Nodal sections were stained in Foster's tannic acid–iron chloride–safranin and also in Cross' safranin-fast green as outlined by Brooks *et al. (3)*. Sections of flower buds, flowers, and fruits were stained by the same safranin-fast green technique and by Mayer's hemalum *(12)*.

To facilitate sectioning, nodes and fruits 10 days after anthesis and older were partially softened by soaking overnight in a mixture of one part glacial acetic acid and nine parts 70 percent ethanol *(4)*.

Statistical treatment of data following a binomial distribution was analyzed by the 95 percent confidence interval method *(14)*. This yielded the interval range between a lower and an upper limit for the mean of each treatment and was used in tables 2 and 3 to determine significant differences between the means.

EXPERIMENTAL RESULTS

Floral Bud Differentiation and Development

Floral bud differentiation on primary branches in active vegetative growth occurred as early as 8 to 10 days before the first flower buds emerged in the leaf axils (fig. 2). In a few instances flower buds were produced in the leaf axils of the node bearing the youngest pair of fully expanded leaves. The first stem from the left in figure 2 shows such a situation and illustrates the earliness of floral bud

TABLE 1. Floral bud emergence on secondary branches which were induced by pruning primary branches of two clones of acerola

CLONES	MONTH OF PRUNING	DAYS BETWEEN PRUNING AND EMERGENCE OF SECONDARY BRANCHES		DAYS BETWEEN PRUNING AND FLOWER EMERGENCE ON SECONDARY BRANCHES		DAYS BETWEEN PRUNING AND PEAK FLOWERING OF ENTIRE PLANT
		Range	Mean	Range	Mean	MEAN
3A-T8	February	10–45	19	40–52	47	47
Maunawili	February	8–20	12	41–60	51	54
3A-T8	April	4–13	11	15–25	19	14
Maunawili	April	4–15	10	18–34	23	6

emergence after initial differentiation. Although flowering occurred in the leaf axils of actively growing primary branches, heaviest flowering was produced in leaf axils of numerous secondary branches. These branches were characterized by very short internodes, resembling rosettes, and were the branches where mature fruits normally occurred.

Vegetative axillary bud development was induced by pruning primary branches 20 centimeters from the apex. Emergence of the secondary shoots was mainly confined to the first two or three nodes below the site of decapitation and occurred as early as 4 days after pruning (table 1). The sequence of emergence of the axillary shoots was basipetal from the cut end. In a few instances emergence of two secondary shoots, one above the other, was observed at single leaf axils of the uppermost nodes of the primary branch. Figure 3 illustrates clearly the presence of numerous axillary meristems in a single leaf axil.

The time between initiation of floral bud differentiation on the secondary branches and the peak in a single flowering cycle of the whole plant were closely related. Data in table 1 show that there was a longer period of time between pruning of primary branches and production of flowers on secondary branches, when pruning was done in February (during nonflowering period) than in April (beginning of flowering period). Induced secondary branches from the February pruning produced tertiary rosette-like spurs in the leaf axils and the secondary branches resembled the unpruned primary branches when flowering occurred in April.

Emergence of the first flower buds from the April pruning was observed in the lowest leaf axils on those secondary branches which emerged first. Flower bud emergence was detected 15 days after pruning in Clone 3A-T8 and 18 days after pruning in Clone Maunawili. The former was pruned 14 days prior to peak flowering of the entire plant and the latter 6 days before the peak in its flowering cycle.

Anatomical preparations of nodes made during a flowering period showed the various stages of floral bud differentiation, illustrated in figures 3 to 8. Axillary

buds originated in partly vacuolated tissue which appeared to contain numerous axillary meristems. Figure 3 shows the superposed arrangement of the meristems, the uppermost of which is the oldest. Figure 4 shows a dome-shaped vegetative apical meristem possessing a tunica-corpus organization. This meristem is subtended by opposite leaf primordia. The delimitation between the tunica and the corpus was not always clear since the outer layer of corpus cells was regularly arranged.

As flowering was initiated, the apical meristem of the axillary shoot ceased to produce leaf primordia and gradually flattened out at the apex. The phenomenon was accompanied by cellular enlargement and increased vacuolation. The sepals, petals, stamens, and carpels were differentiated in acropetal sequence (figs. 5 to 7). Clear differentiation of these structures occurred by the time the flower bud emerged in the leaf axil (fig. 8).

Development of Ovule

There was much variation in ovule development in the three clones studied. The first type of development described here is what may be interpreted to be a normal ovule development from its inception to fertilization.

Normally, a single ovule was differentiated in each locule by the time the flower bud emerged from the leaf axil, 6 to 7 days before anthesis in the three clones studied. The inner integument was present as a short ring of tissue extending to about half the length of the nucellus (fig. 9). This figure also shows a single sporogenous cell. The tip of the integument was directed towards the receptacle end of the flower bud at this stage.

The outer integument was differentiated as an outer ring of tissue before curvature of the developing ovule was observed (fig. 10). Unequal growth of the funiculus caused the ovule to turn away from the receptacle end of the bud, becoming semi-anatropous 4 or 5 days prior to anthesis (fig. 11). Rapid growth of the nucellus and outer integument occurred simultaneously with curving of the ovule so that the nucellus extended well beyond the inner integument, forming a nucellar beak. This is clearly illustrated in figure 11. The long outer integument enclosed the inner integument and nucellus. The ovule almost filled the locule at 3 days before anthesis and some completely filled locules were also observed. The micropylar canal occurred in folds over the nucellus or was more or less straight. While the inner integument continued growing slowly, the micropyle at anthesis was formed by the outer integument only. The cells of the nucellus began disintegrating from the chalazal end 2 days after anthesis and the embryo sac became pear-shaped with increase in size. Figure 12, illustrating the ovule on the day of anthesis, shows the disintegration of nucellar cells at both ends.

Abnormal development of the ovule in which cells of the nucellar beak began disintegrating was observed in flowers collected on the day of anthesis. Counts of 36 ovules from 12 ovaries of Clone A at anthesis yielded 33.3 percent of ovules, or 1.0 ovule per ovary having this abnormality. The disintegrating cells shown in figures 12 to 16 were much larger than the other nucellar cells and had thinner

walls. Many of the larger cells were characterized by large vacuoles before disintegrating. At 3 days after anthesis, the nucellar cells in this type of abnormality were almost completely disintegrated and abortion of the embryo sac was observed (fig. 13). Figure 16 shows evidence of disintegrated nucellar cells at the chalaza and also what appeared to be two pollen tubes that have penetrated the micropyle. Fertilization cannot possibly occur in these types of ovules. The disintegration of nucellar cells before fertilization indicate strongly a cause of low seed set in acerola.

Formation of other abnormal ovules are shown in figures 17 to 23. In some of the cases studied, two ovules were differentiated in some locules early in floral development (fig. 17). This phenomenon was found in Clones B and C, but not in Clone A. To determine the frequency of this abnormality and the subsequent development of the abnormal ovules, carpels of 100 mature fruits of each of the three clones were examined. Clone A showed no abnormality, each locule having only a single ovule. Clone B showed 50 percent of its fruits with two ovules per locule but this occurred only in one of three locules per fruit. Locules of fruits of Clone C show that 72 percent had one ovule, 24 percent had two ovules, and 4 percent had two each within two of the three locules.

None of the locules bearing two ovules contained fully formed seeds at maturity. The ovules had aborted and were collapsed. Figure 18 shows the orientation of the two ovules from a young ovary 3 days after anthesis. The micropyle of ovule *a* is at the basal end of the locule. That of the anatropous ovule *b* points between the funiculus and placenta. The orientation of both ovules appears to be such that pollen tube penetration would seem unlikely in most instances. Frequency of occurrence of two ovules in one locule of an ovary, the abnormal orientation of double ovules, and the aborted ovules in mature fruits appear to be contributing factors in low seed set in acerola.

Further evidence of abnormal ovule orientation in relation to pollen tube growth is shown in figures 19 and 21. Figure 21 shows a semi-anatropous ovule, but the micropyle occurs between the axil of funiculus and placenta. Figure 19 shows two locules, each of which contains an inverted ovule with the micropyle opposite the receptacle end of the fruit. Counts of 84 ovules within 28 ovaries of

PLATE II

FIGURE 7. Development of ovules *o* in relation to pistil *b*. *s* shows well-developed stamens. (58×)

FIGURE 8. Flower bud on the day of emergence. Single integument is shown as a short ring of cells *o*. Micropyle of ovule is adjacent to the receptacle end of flower bud. Anther is indicated by *a*. (30×)

FIGURE 9. Single sporogenous cell *s* at 6 days before anthesis. Short integument *i* occurs at this stage. (740×)

FIGURE 10. Young ovule with single sporogenous cell *s* and showing differentiation of outer integument *e* and inner integument *i*. (149×)

FIGURE 11. Extension of nucellus beyond inner integument *i* forming a nucellar beak *n*. Outer integument *e* is longer than nucellus. Ovule is semi-anatropous. Funiculus *f* and a two-nucleate sporogenous cell *m* are shown. (291×)

FIGURE 12. Ovule abortion on the day of anthesis. Disintegration of nucellar cells in this figure is shown at both the micropylar and chalazal ends. (85×)

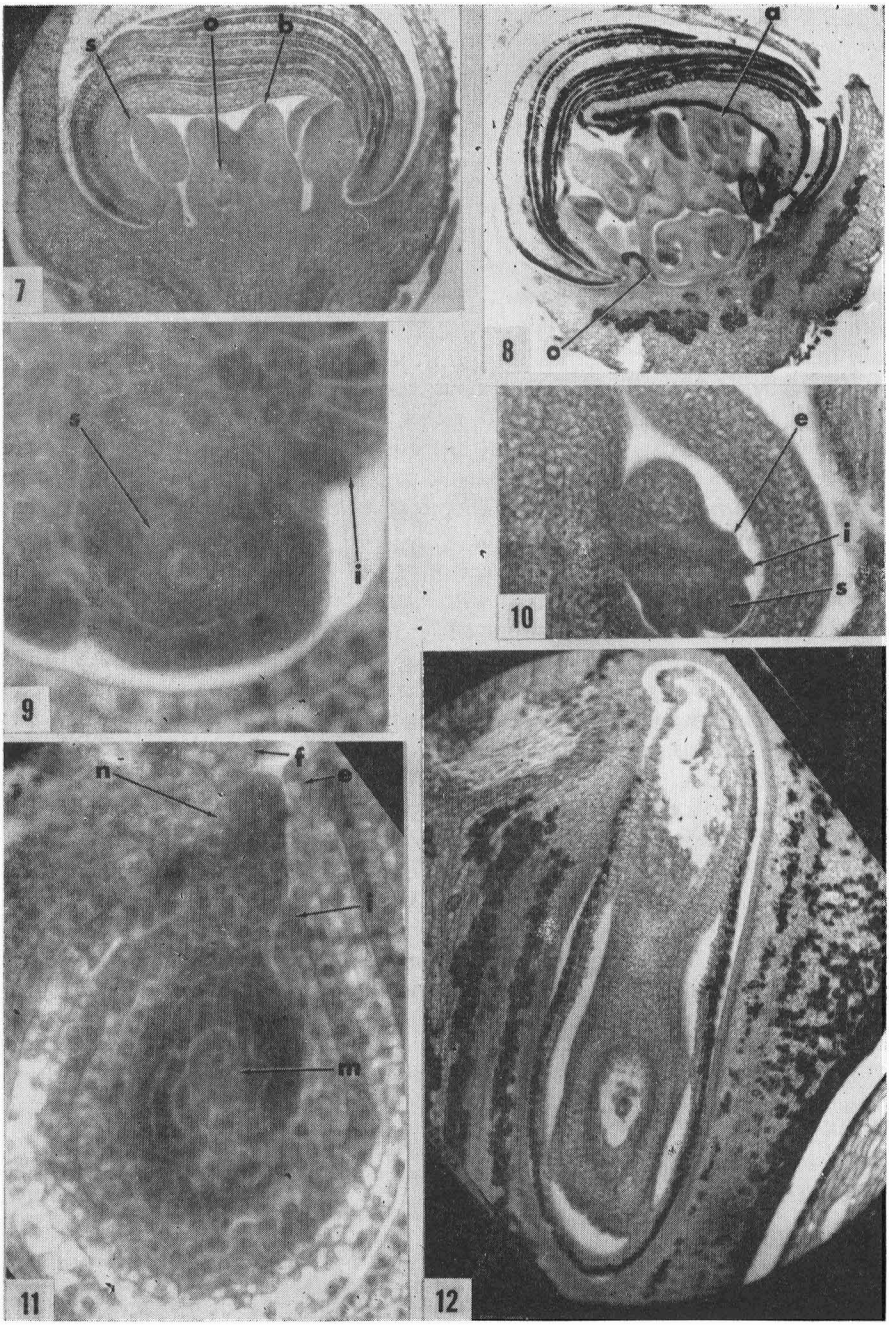

PLATE II

Clone A showed that the frequency of inverted ovules was 8.3 percent, or 0.25 per ovary.

Megagametogenesis and Embryo-Sac Development

The development of the embryo sacs within the ovules showed much variation and abnormality in all three clones studied. The sporogenous cells of flower buds 6 to 7 days prior to anthesis consisted of either a single large megaspore mother cell (fig. 9) or showed several sporogenous cells of different sizes and arrangements (figs. 24 and 25). Figure 24 shows three sporogenous cells, two of which are shown clearly and one rather faintly. In figure 25 two sporogenous cells can be seen in the photograph. The larger megaspore mother cell appeared to be the only functional one.

Normally, only a single sporogenous cell developed within an ovule and formed a 16-nucleate mature embryo sac of the *Penaea*-type. However, in one ovule of a bud collected 3 days before anthesis, two developing embryo sacs were observed. This is clearly shown in the ovule on the right in figure 23. Two embryo sacs are found one above the other. They may have originated from two adjacent megaspore mother cells.

Nuclear division of the megaspore mother cell occurred at various times. The first reduction division was observed 4 or 5 days prior to anthesis (fig. 26). The cytoplasm between the two resulting nuclei in the megaspore mother cell appeared fibrillar. Megasporogenesis was completed 3 to 4 days before anthesis when a four-nucleate cell was present. Each of the nuclei is interpreted to be a megaspore. These four nuclei were situated towards the periphery of the embryo sac (fig. 27). Just before anthesis, additional nuclear division occurred, giving rise to a 16-nucleate embryo sac. The 16 nuclei were arranged in four groups of 4 nuclei each (fig. 29) and were enclosed in peripheral cytoplasm. Figures 28 and 29 are composite camera lucida drawings prepared from serial sections of embryo sacs.

Upon maturity, the embryo sac consisted of five nuclear groups. Four peripheral groups each contained three nuclei, while the central four-nucleate polar group was formed after migration of one nucleus from each of the original peripheral groups (fig. 30). The embryo sac appeared mature on the day of anthesis. Polarity in embryo-sac development seemed weak and there was considerable variation in

PLATE III

FIGURE 13. Disintegration of nucellus and embryo-sac abortion. (84×)
FIGURE 14. Ovule and nucellar disintegration on the day of anthesis. (72×)
FIGURE 15. Disintegrated nucellus at 3 days after anthesis. (72×)
FIGURE 16. Disintegrated ovule at 3 days after anthesis. Two pollen tubes are shown faintly at the micropyle at *p*. Embryo sac has completely disintegrated as indicated by *e*. (80×)
FIGURE 17. Cross section of ovary showing two ovules *o* in one locule and single ovule each in the other two locules. (59×)
FIGURE 18. Two ovules per locule showing abnormal positions of the micropyles indicated by the letter *m*. That of one ovule *a* is adjacent to the receptacle end of the locule and that of the other ovule *b* is semi-anatropous but faces the attachment of funiculus and placenta *f*. (35×)

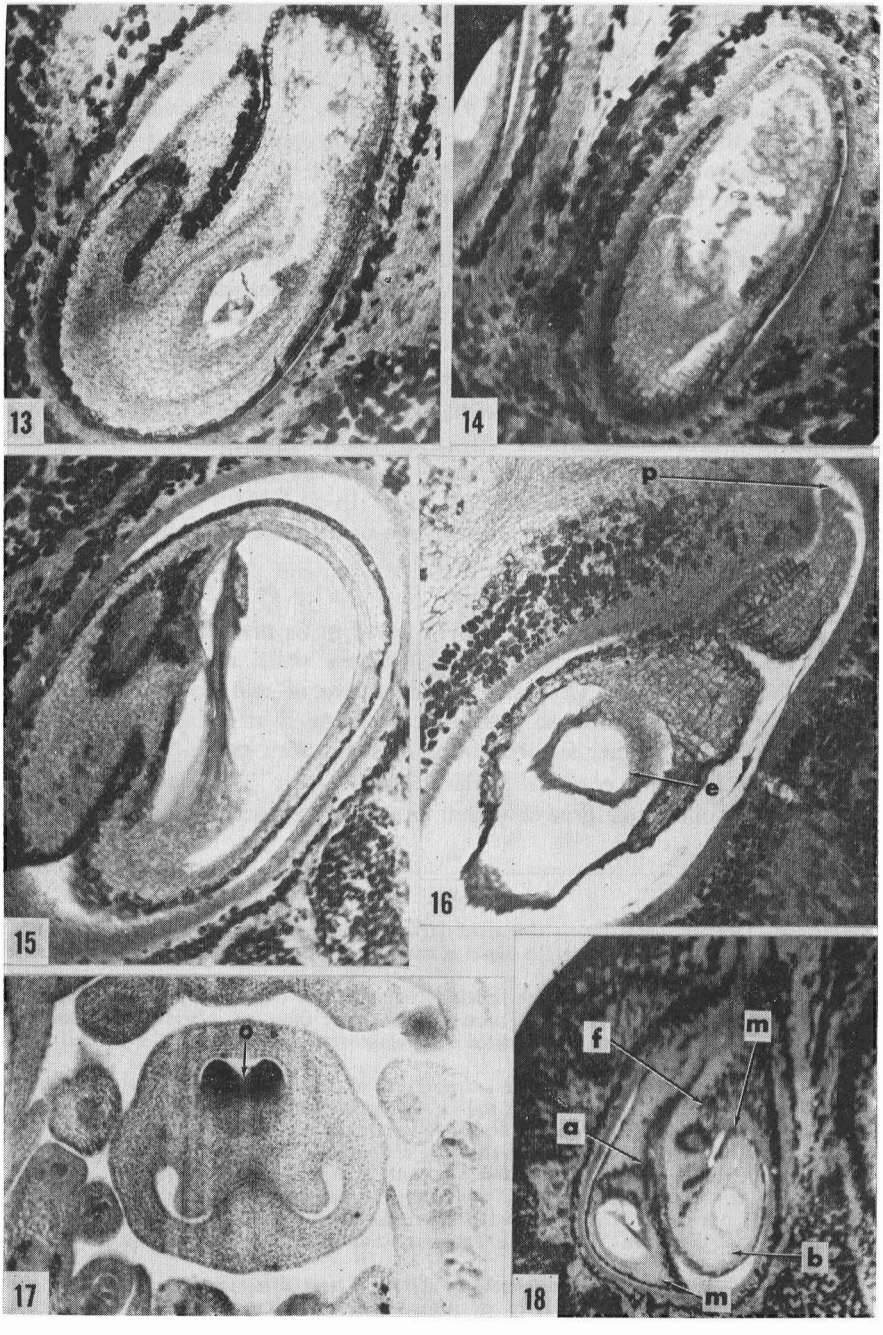

PLATE III

nuclear arrangement. The nuclei of the mature embryo sac were approximately the same size and the egg was not morphologically distinguishable.

The embryo sac occurred in various places within the nucellus. Figures 20, 21, and 31 indicate that the embryo sac at anthesis may lie deep within the nucellus or closer to the micropyle. Usually, however, the embryo sac at anthesis was situated deep within the nucellus. The location of the embryo sac within the nucellus might be a determining factor influencing the time and success of fertilization. The micropylar end of the embryo sac at anthesis was enlarged and the sac appeared to be pear-shaped. Further enlargement of the micropylar end occurred and the sac in some ovules at 3 days after anthesis showed a sharply constricted chalazal end.

Various types of abnormalities from nucellar disintegration to ovule abortion were frequently observed during embryo-sac development in the three clones studied. Figures 12 to 16 show those abnormalities that may ultimately result in ovule sterility. The abnormalities ranged in time of appearance from very early in megasporogenesis where the megaspore mother cell failed to undergo meiosis, to later stages when nuclei disintegrated (figs. 13 and 16). These abnormalities undoubtedly contribute to the low percentage of seed formation in acerola.

Development of Anthers and Pollen

Development of anthers and pollen appeared to be normal in all three clones studied. However, two exceptions were observed which may be considered to occur rather infrequently. One was the occurrence of numerous aborted or collapsed pollen grains within one anther sac of Clone B at 3 days before anthesis (fig. 32). Adjacent anther sacs contained normal pollen grains.

The other exception observed is illustrated in figure 33. It shows pollen germination and pollen tube growth within an undehisced anther sac of Clone A at

PLATE IV

FIGURE 19. Two locules showing abnormal positions of ovules. Ovule in the left locule is upside down and the one in the right ovule is curved toward the attachment of funiculus and placenta. (24×)

FIGURE 20. Embryo sac situated at the chalazal end of the ovule. Nucellus shows constricted central portion and may abort later. Embryo sac *e* shows three peripheral nuclei; the more densely stained central portion may contain disintegrated nuclei. *c* shows the chalazal end of the ovule. (85×)

FIGURE 21. Embryo sac *e* situated at micropylar end of ovule. The pollen tube is interpreted to grow down the ovary at *p* and enters the locule to the right of the funiculus *f*. The micropyle *m* is shown between the axil of funiculus and placenta. (75×)

FIGURE 22. Aborting ovule at anthesis showing disintegration of nucellus *n* and degeneration of nuclei in the embryo sac *d*. Constriction *c* of ovule is shown. (75×)

FIGURE 23. Abnormal development of two ovules in one locule. The left ovule contains a single sporogenous cell *s*. The right ovule shows the development of two embryo sacs *e* arranged one above the other. The outer integument *i* and micropyle *m* are facing the receptacle end of the locule. (121×)

FIGURE 24. Ovule at 6 days before anthesis shows at least three sporogenous cells *s*. Two are clearly seen while the third is partly out of focus below the other two. (514×)

FIGURE 25. Ovule at 6 days before anthesis shows two sporogenous cells *s*. (75×)

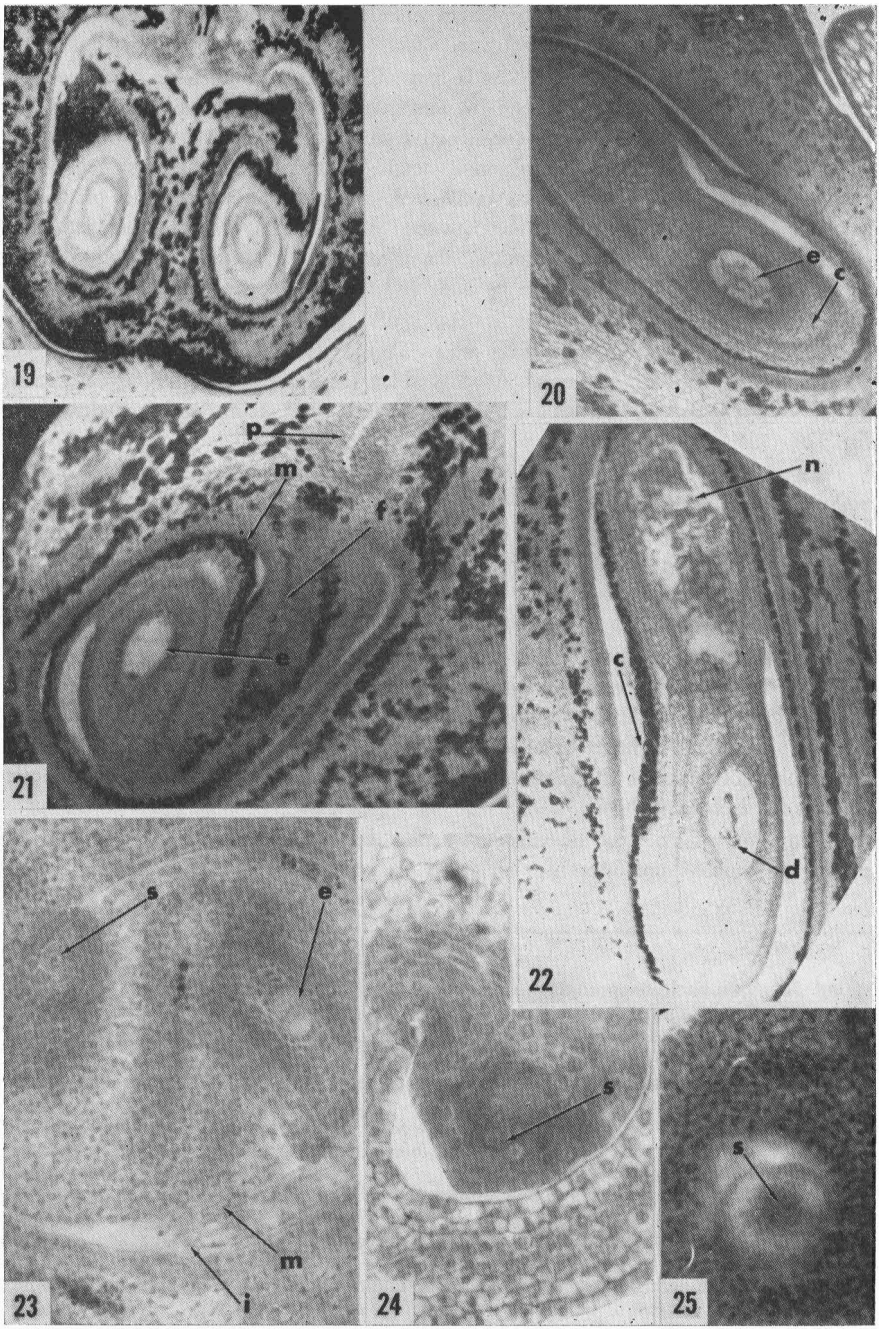

PLATE IV

anthesis. Germination of pollen grains in undehisced anther sac has been reported in cleistogamous flowers by Maheshwari *(7)*.

In cross section the anthers of buds 6 to 7 days before anthesis appeared as a structure with two large lobes, each of which consisted of two smaller lobes (fig. 34). Each of these smaller lobes contained a single pollen sac (microsporangium) in which several layers of sporogenous cells (pollen mother cells) formed a central cylinder. The sporogenous cells were surrounded by a layer of radially elongated tapetal cells, outside of which were two middle layers and an endothecium and epidermis. Single microsporangia are illustrated in figures 35 and 36. The two larger lobes were located side by side and adaxial to the connective.

Reduction division of pollen mother cell showing a two-celled stage was observed 5 days before anthesis (fig. 35). Tetrads of microspores (fig. 36) were observed 4 days before anthesis. At this time the tapetum began disintegrating and at 3 days before anthesis the tapetal cells had completely distintegrated (fig. 37).

Pollen grains formed by the separation of the tetrads of microspores possessed single nuclei at 3 days before anthesis, but had increased in size and had developed reticulately thickened walls. A second nucleus was observed in each pollen grain at 2 days before anthesis. The pollen grain at this stage was morphologically indistinguishable from the mature binucleate grain at anthesis.

The four pollen sacs remained distinct until 3 days before anthesis. At this time growth of the anther forced the movement of the two larger anther lobes to slightly lateral positions in respect to the connective. Two days before anthesis, the partition separating the two pollen sacs within each larger anther lobe ruptured (fig. 38), and disintegrated completely the day before anthesis. Thus, each larger anther lobe contained a single chamber filled with morphologically mature pollen grains 1 day before anthesis.

Normally, pollen was liberated on the day of anthesis. However, anther dehiscence showed considerable variation in both degree and time among the

PLATE V

FIGURE 26. Two-nucleate sporogenous cell *m* at 4 days before anthesis. (263×)
FIGURE 27. Four-megaspore stage at 3 days before anthesis. (376×)
FIGURE 28. Camera lucida drawing of an eight-nucleate embryo sac at 3 days before anthesis. (621×)
FIGURE 29. Camera lucida drawing shows a 16-nucleate embryo sac at 2 days before anthesis. (481×)
FIGURE 30. Formation of polar nuclear group at 1 day before anthesis. Two peripheral nuclear groups are shown and one nucleus *n* from each group appears to be migrating toward the center of the embryo sac. (134×)
FIGURE 31. Embryo sac situated at center of nucellus. Chalaza *c* and embryo sac *e* are shown by arrows. (37×)
FIGURE 32. Numerous aborted pollen grains in anther sac of Clone B 3 days before anthesis. (220×)
FIGURE 33. Pollen germination in an undehisced anther of Clone A at anthesis. (151×)
FIGURE 34. Cross section of anther at 6 days before anthesis. There are two larger lobes *L* and each larger lobe consists of two smaller lobes *l* adaxial to the connective *c*. (67×)

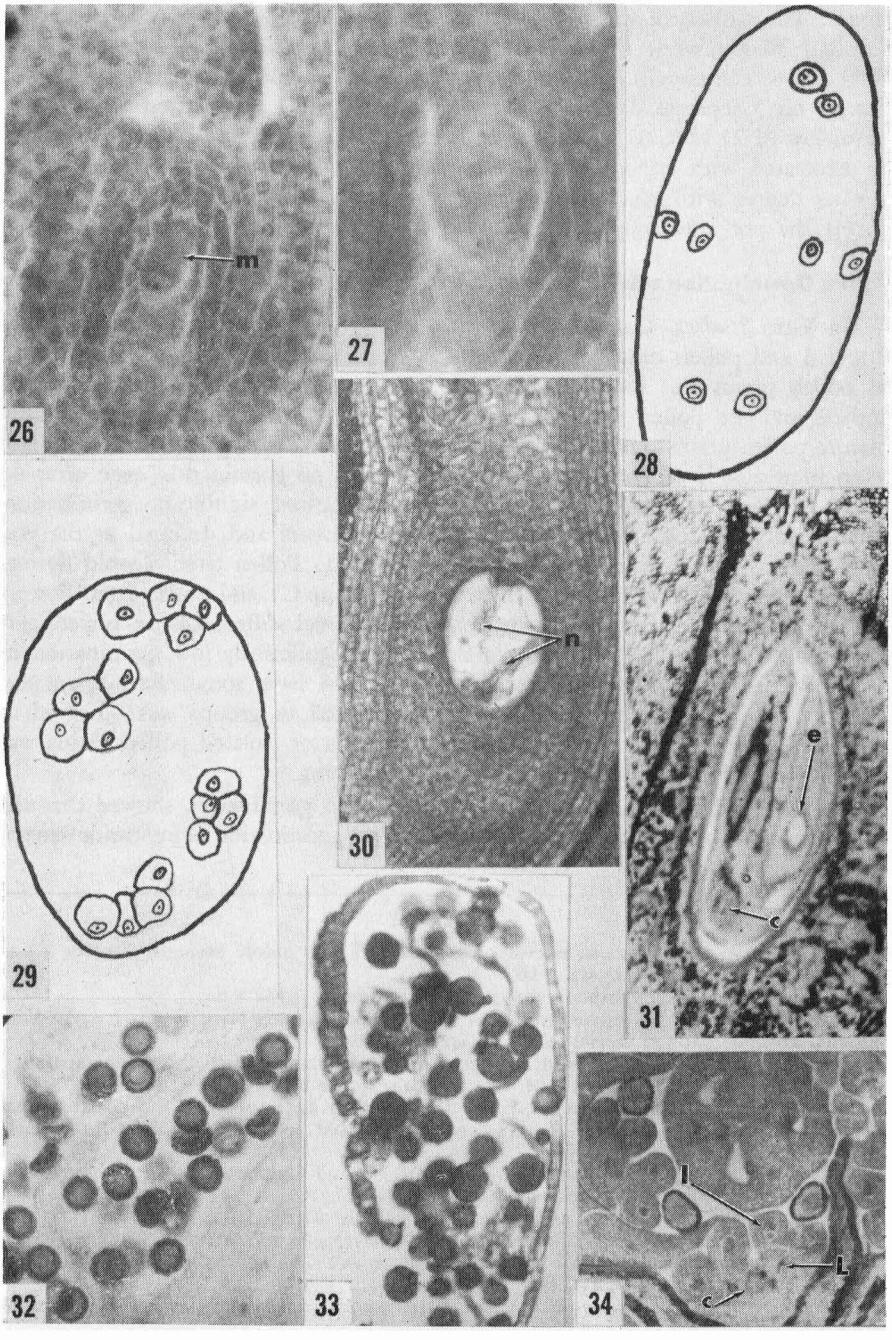

PLATE V

clones. The anthers of Clone C normally dehisced by 6:30 AM, those of Clone A before 7:30 AM, while the anthers of Clone B either initiated dehiscence around 1:00 PM or completely failed to dehisce in observations made of newly opened flowers on 5 separate days. The temperature range for this period was from a mean low of 21.1° C to a mean high of 30.8° C. Anther dehiscence appeared to be associated with atmospheric conditions, particularly with temperature and to a lesser degree with precipitation. Higher morning temperature with some rains during the previous evening seemed to promote anther dehiscence.

Pollen Germination and Pollen Tube Growth

In Vitro *Studies.* Data on pollen germination *in vitro* are presented in tables 2 and 3 and pollen tube growth is illustrated in figures 39 to 41. Germination of pollen grains was found to be related to the time of natural dehiscence of anthers and the pollen was germinable for only a short time. Morphologically mature pollen grains removed from the anthers 1 to 3 days prior to anthesis and sown immediately on sucrose-agar medium showed no germination even after 48 hours. Pollen from naturally dehisced anthers showed significant germination. Peak germination occurred best in the afternoon hours and declined at the end of the day as shown in table 2 and figures 39 to 41. Pollen from day-old flowers showed almost no germination (2.3 percent for Clone C) and pollen from flowers 2 days after anthesis showed no germination. Clonal differences in pollen germination were also observed as indicated by the significantly low germination in Clone B (table 2). Density of pollen appeared to have some effect upon germination rate, as germination of pollen congregated in groups was observed as early as 30 minutes after sowing, while germination of isolated pollen grains was not observed until approximately 2 hours after sowing.

Studies on the effect of stylar extracts on pollen germination showed that the addition of stylar extracts did not increase pollen germination significantly except

PLATE VI

FIGURE 35. Two-celled stage in microsporogenesis at 5 days before anthesis. Dydads *d* and tapetal cells *t* are shown by arrows. (165×)
FIGURE 36. Tetrads of microspores at 4 days before anthesis. (148×)
FIGURE 37. Separation of microspores from tetrads in pollen grain formation at 3 days before anthesis. Disintegration of tapetal cells *t*. (206×)
FIGURE 38. Anther at 2 days before anthesis showing rupture *r* of the partition separating the two smaller lobes of the larger lobe. Connective is shown by the letter *c*. (102×)
FIGURE 39. Bursting of pollen tubes using distilled water as the solvent in the sucrose-agar medium. Pollen grains of Clone A were sown at 7:30 AM and photographed 5 to 6 hours later. (75×)
FIGURE 40. Pollen grains of Clone A sown at 1:30 PM and photographed 5 to 6 hours later. Shows germinated pollen grains with long pollen tubes. (75×)
FIGURE 41. Pollen germination and pollen tube growth of Clone A sown at 7:30 PM and photographed 5 to 6 hours later. Shows considerable decrease in germination. (75×)
FIGURES 42 AND 43. Chemotropic responses in growth of Clone C pollen tubes toward the stigmas and styles of Clone B in culture. (75×)
FIGURE 44. Pollen tube *t* growth of Clone S pollen *p* through excised style of Clone B. (75×)

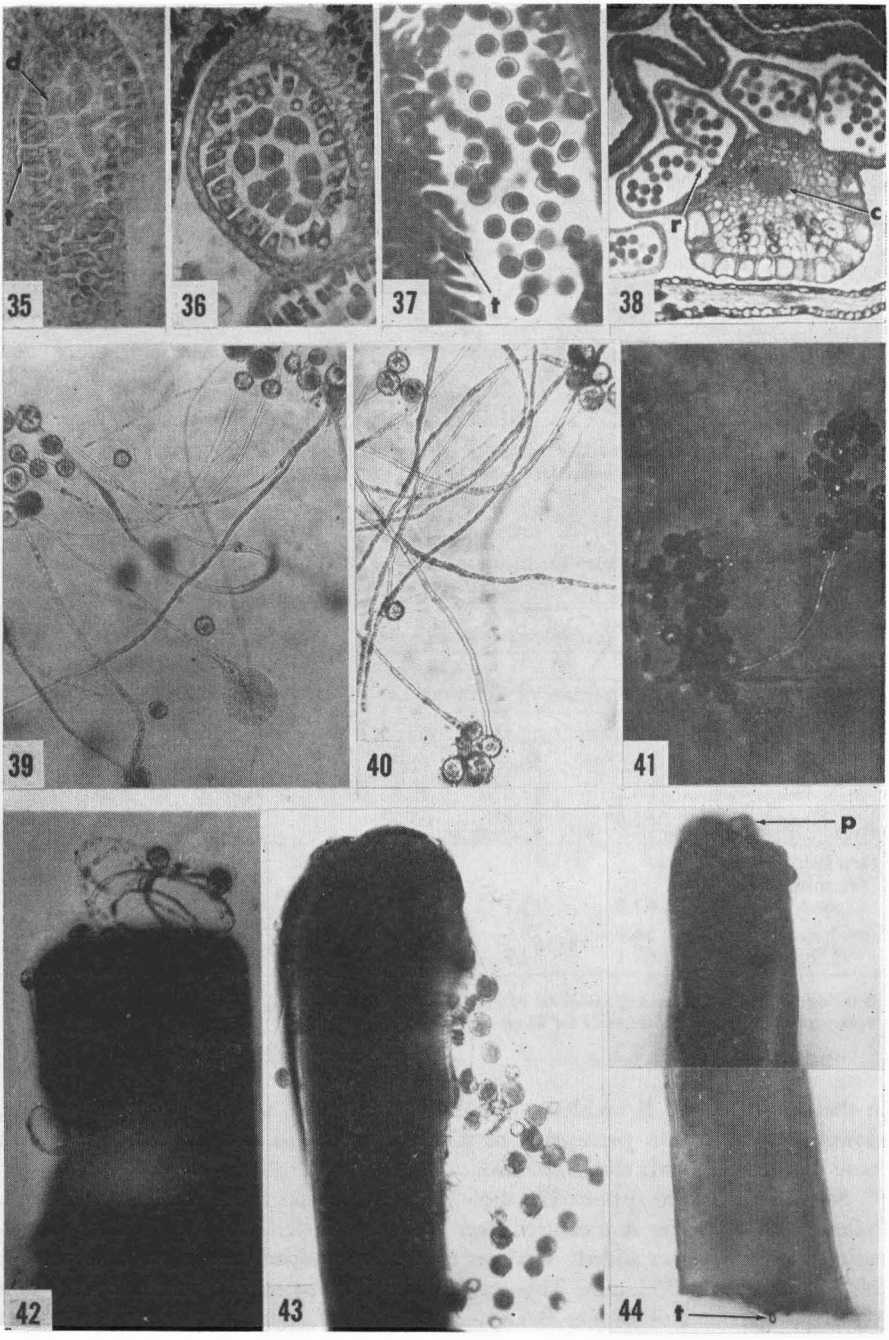

PLATE VI

TABLE 2. Percentage of pollen germination of three acerola clones 5 hours after sowing on the day of anthesis

CLONES	TIME OF ANTHER DEHISCENCE	TIME OF SOWING 7:30 AM	1:30 PM	7:30 PM	MEAN PERCENTAGE GERMINATION OF CLONES	CONFIDENCE LIMITS (95% LEVEL)
		Percentage of Germination				
A	7 AM	37.9*†	62.6*†	32.0*	47.0	39.6–54.4
B	1 PM	00.0*†	30.2*†	7.7†	11.1*	7.1–15.8
C	6 PM	55.6†	67.8*†	37.2*†	50.2	44.0–56.0
Mean Percentage Germination of Treatments		14.8†	53.7†	26.8†		
Confidence Limits (95% Level)		9.5–21.0	48.1–59.9	21.2–32.8		

* Significantly different percentage values for clones.
† Significantly different percentage values for time of sowing.

TABLE 3. Percentage of pollen germination with the addition of stylar extracts on the day of anthesis of three acerola clones

STYLAR EXTRACTS OF CLONES	POLLEN OF CLONES A	B	C	MEAN PERCENTAGE GERMINATION WITH EXTRACTS	CONFIDENCE LIMITS (95% LEVEL)
	Percentage of Germination				
A	56.5	32.4*	64.6*	52.7	43.8–62.2
B	67.6*	50.0†	71.9*	64.2	47.1–67.5
C	64.7	43.6*	69.4	59.8	50.6–69.4
None	62.6*	30.2*	67.8*	55.0	49.0–61.0
Mean Percentage Germination of Clones	62.5	37.2†	67.7		
Confidence Limits (95% Level)	54.9–69.1	29.6–44.9	61.2–74.6		

* Significantly different percentage values for effects of stylar extracts.
† Significantly higher percentage value for Clone B pollen.

in the case of Clone B (table 3), which normally had a lower germination rate. However, germination percentage of Clone B was still significantly lower than those of the other two clones studied.

Stylar extracts also appeared to show inhibitive effects upon pollen tube growth. Pollen tubes of Clone A measured over 1.80 mm in length 5 hours after germination with no extract added. With its own extract added, maximum tube growth was approximately 0.71 mm. This was further reduced to 0.15 mm and 0.50 mm with stylar extracts of Clones B and C, respectively. Similar effects upon pollen

tube growth were observed in the other two clones. Weakest effects of inhibition were exhibited when extracts and pollen were from the same clone. Stylar extracts of Clone B which showed beneficial effects upon germination of its own pollen appeared to show strongest inhibitive effects upon pollen tube growth of Clones A and C.

In vitro studies of pollen germination and tube growth indicated presence of a mild chemotropic response wherein growth of pollen tubes appeared to be directed towards the stylar segments (figs. 42, 43). Growth was not strongly attracted towards the stigmatic surfaces as might be expected if some attractant or growth-promoting substance was secreted by the stigmas as in certain grasses *(19).*

Stylar segments decapitated 4 hours after pollination and placed on agar showed pollen tubes emerging at the excised base 16 to 17 hours after pollination (fig. 44). The stylar segment measured 1.03 mm in length and the length of the pollen tube at that stage was 1.05 mm. Measurements of length of 15 styles from the stigmatic tip to the point of attachment on the ovaries yielded an average length of 3.26 mm for Clone B. At this rate of growth, the pollen tubes would take approximately 2 to 3 days to grow through the entire length of the style. *In vivo* studies presented below support this observation.

In Vivo *Studies.* Pollen tube growth and the pathway of tube penetration were studied in hand-pollinated flowers. It was observed that pollen germination and tube penetration into the styles occurred within 4 hours after pollination in the three clones studied. At 24 hours after pollination the pollen tubes had penetrated approximately one-third to one-half the length of the styles. By 50 hours after pollination, the pollen tubes had entered the locules. Figures 45 and 46 show pollen tube growth in relation to the ovule. Figure 47 shows what is interpreted to be the normal pathway of pollen tube growth into the ovule. It also shows the top of the ovary immediately below a style. The pollen tubes *(p)* are shown growing into a tissue with large intercellular spaces and heading laterally toward the micropyle *(m),* which lies adjacent to the pathway of the growth of the pollen tubes. Figure 46 shows a pollen tube *(t)* penerating the micropyle *(m)* in the next section of the ovary from which figure 47 was made.

What appeared to be pollen tube penetration into the embryo sacs of Clones A and C was observed 2 to 3 days after pollination (fig. 48). The pathway of the pollen tube was indicated by darkly stained cells and the pollen tube seemed to be disintegrating. Pollen tube penetration into the embryo sac of Clone B occurred 2 days after pollination. There were no signs of pollen tubes near the embryo sac in sections made 24 hours after pollination. A two-nucleate proembryo stage (fig. 49) was seen 72 hours after pollination.

The differences in time of pollen tube penetration into the embryo sacs may be attributed to the differences in location of the embryo sacs within the nucellus. Measurements of the relative distance between the tip of the outer integument (micropyle) and the tip of the embryo sac wall, perpendicular to its long axis at the micropylar end, of five ovules each at 3 days after anthesis showed average distances of 0.45 mm, 0.52 mm, and 0.56 mm for Clones B, A, and C, respectively.

Results of this study seemed to indicate that absence of pollination, rather than failures in pollen germination and pollen tube growth, is a contributing factor to poor fruit and seed set in acerola.

Development of Fertilized Ovules

Double Fertilization. Signs of double fertilization were observed 2 to 3 days after pollination (figs. 48, 50). Embryo sacs at 4 days after pollination either showed signs of disintegration or contained proembryos with or without primary endosperm cells. In figure 48, which shows an embryo sac 2 days after pollination, a pollen tube *(t)* appears to be freeing two sperm nuclei *(s)*. The egg *(e)* may be located at the micropylar end and what seems to be a binucleate endosperm mother cell *(m)* occurs at the chalazal end of the sac. Two other polar nuclei appear to have fused but seem to be disintegrating *(d)*.

In figure 50, which illustrates an embryo sac 3 days after pollination, structures interpreted to be the egg and the endosperm mother cell are shown at *e* and *m*, respectively. The egg nucleus seems to be attached at the chalazal end of a large suspensor-like cell, which in turn seems to be attached to the embryo-sac wall at the micropylar end. The two darkly stained "spots" *(s)*, which are interpreted to be the two sperm nuclei, have migrated separately, one towards the endosperm mother cell *(m)* and the other adjacent to the egg cell *(e)*, thus initiating double fertilization.

A few embryo sacs collected from flowers which were open for 3 days and even longer contained as many as nine groups of what appeared to be free nuclei (fig. 51). The number of nuclear groups and the number of nuclei within groups varied. The nuclear groups were connected by densely stained cytoplasm and showed variations in their arrangements in all three clones.

The origin and significance of these groups of nuclei are not clear. It was

PLATE VII

FIGURE 45. Pollen tubes *t* did not enter the ovule *o* and have burst at the receptacle end of the locule. (60×)

FIGURE 46. Pollen tube *t* penetration into the micropyle *m* 3 days after hand pollination at anthesis. (474×)

FIGURE 47. Normal pathway of pollen tube growth *p* into the ovary and locule are shown by arrows. The micropyle *m* lies adjacent to the pathway. (107×)

FIGURE 48. Embryo sac 2 days after pollination and containing a pollen tube *t* which has burst at the chalazal end of the sac. What may be two sperm nuclei *s* are shown among the contents of the pollen tube. The egg *e* is slightly out of focus and is situated at the micropylar end of the embryo sac. What appear to be a binucleate endosperm mother cell and two other nuclei which might be the polar group of nuclei are indicated by arrows with *m* and *d*, respectively. (356×)

FIGURE 49. Two-celled proembryo stage at 3 days after pollination. The primary endosperm cell has divided forming two cells *e*. The proembryo cell *p* adjacent to the suspensor is flattened and later gives rise to the embryo. The nucleus within the suspensor *s* does not undergo further division and becomes part of the suspensor. (561×)

FIGURE 50. Embryo sac 3 days after pollination. The embryo sac is pear-shaped and may be at the stage of double fertilization. The egg *e* and endosperm mother cell *m* are shown and what may be two sperm nuclei *s* are represented by the lightly stained "spots." One of the sperm nuclei has just about reached the egg. (246×)

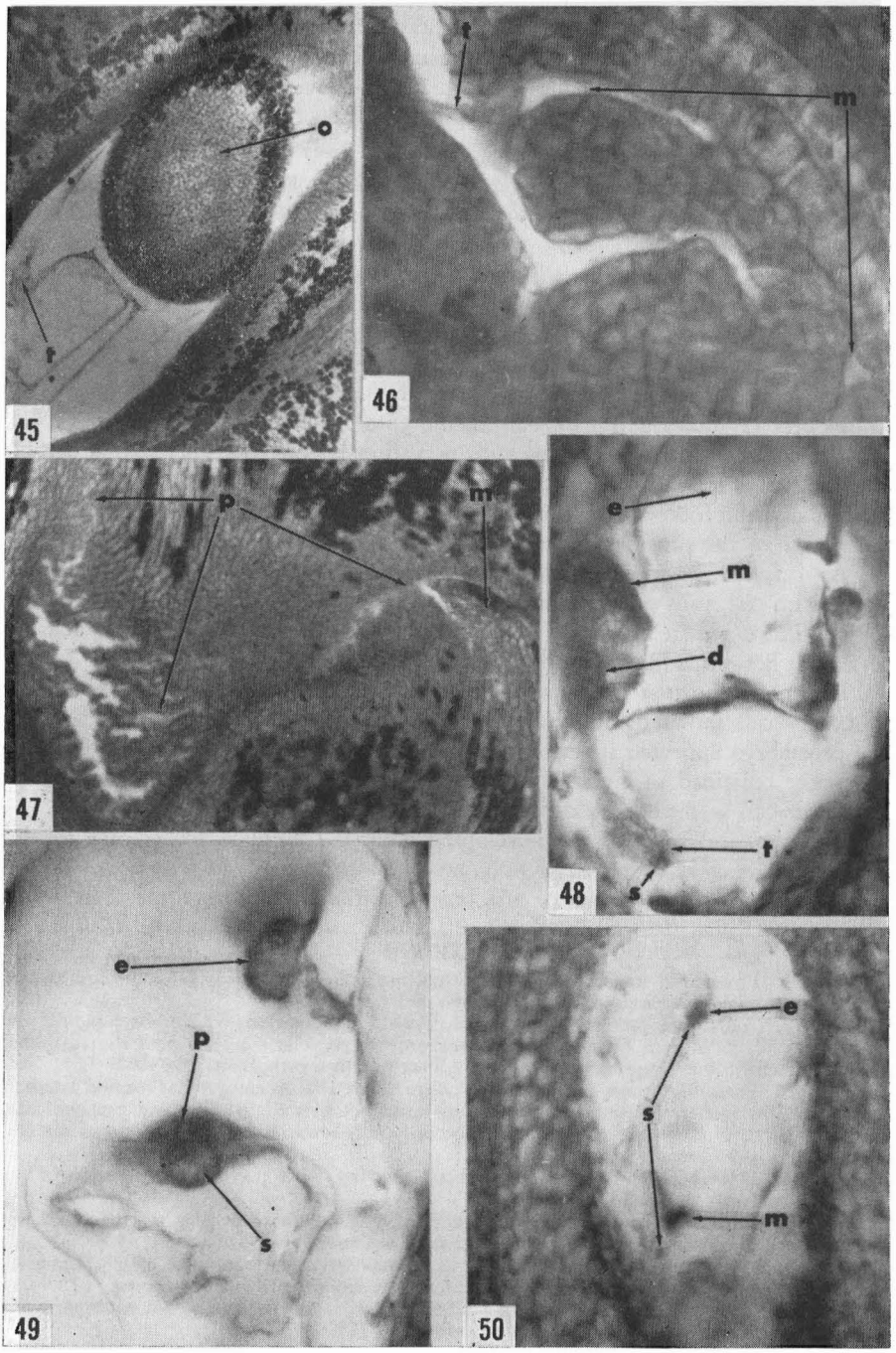

PLATE VII

noted that disintegration of the groups without further division occurred at later proembryo stages. Endosperm development was not observed in embryo sacs containing both proembryo and the nuclear groups. Disintegration of chalazal nucellar cells seemed to be associated with this type of abnormality. Free nuclear groups also occurred in embryo sacs without proembryos.

Embryogenesis and Development of Endosperm. Reference is made here to figure 49, which shows an embryo sac 3 days after pollination. The fertilized egg has divided, forming two cells. A large suspensor cell is present adjacent to the embryo sac wall at the micropylar end. The suspensor nucleus *(s)* occurs within the suspensor cell at its chalazal end. The proembryo *(p)* is adjacent to the suspensor nucleus *(s)* but occurs outside of the suspensor wall. Both nuclei at this stage appear to be approximately the same size. The proembryo cell *(p)* later divides and gives rise to the embryo.

Figure 52 shows a four-celled proembryo stage with a large suspensor cell lying adjacent to three proembryo cells occurring in one row, enclosed in densely stained cytoplasm. This arrangement of the four cells very probably arose from nuclear division followed by formation of cell walls perpendicular to the suspensor. This stage of the proembryo occurred 3 days after pollination.

At 4 days after pollination the proembryo showed a second row of cells laid down with cell walls parallel and perpendicular to the long axis of the suspensor. This is illustrated in figure 53. The proembryo is shown to have a rounded top and a flat bottom, adjacent to the suspensor cell.

Figure 54 shows a developmental stage 5 days after pollination. Here, the suspensor cell shows definite attachment to the embryo sac wall. The body of the proembryo appeared spherical and the row of embryonic cells adjacent to the suspensor remained as a connecting row of cells. The single nucleus within the suspensor cell seemed to be disintegrating.

The spherical body of the developing embryo continued to increase in size and cell number at 6 days after pollination (figs. 55 to 57). These figures also illustrate variations in points of attachment of the suspensor to the embryo sac

PLATE VIII

FIGURE 51. Proembryo stage at 4 days after pollination. Free nuclear groups *n* are shown. No endosperm is developing within this embryo sac. (236×)

FIGURE 52. Four-celled proembryo stage at 3 days after pollination. This embryo sac did not show signs of endosperm development. Three proembryo cells *p* adjacent to the suspensor nucleus *s* occur in one row and are enclosed in densely stained cytoplasm. (533×)

FIGURE 53. Proembryo stage at 4 days after pollination. The proembryo has formed a second row of cells by nuclear divisions and the laying-down of cell walls parallel and perpendicular to the long axis of the suspensor. A nuclear group occurs towards the chalazal end of the embryo sac and is indicated by *n*. (249×)

FIGURE 54. Proembryo at 5 days after anthesis showing what may be degeneration of the suspensor nucleus *s*. (197×)

FIGURE 55. Proembryo at 6 days after anthesis showing the attachment of the suspensor *s* at the micropylar end of the embryo sac. Endosperm *e* has developed. (176×)

FIGURE 56. Proembryo at 6 days after anthesis. Suspensor *s* is attached about 923 μ towards the chalazal end of the embryo sac, from the micropyle. Endosperm *e* is developing. (187×)

FIGURE 57. Twin proembryos *p* having a single but enlarged suspensor. No endosperm has developed in this embryo sac at 6 days after anthesis. (212×)

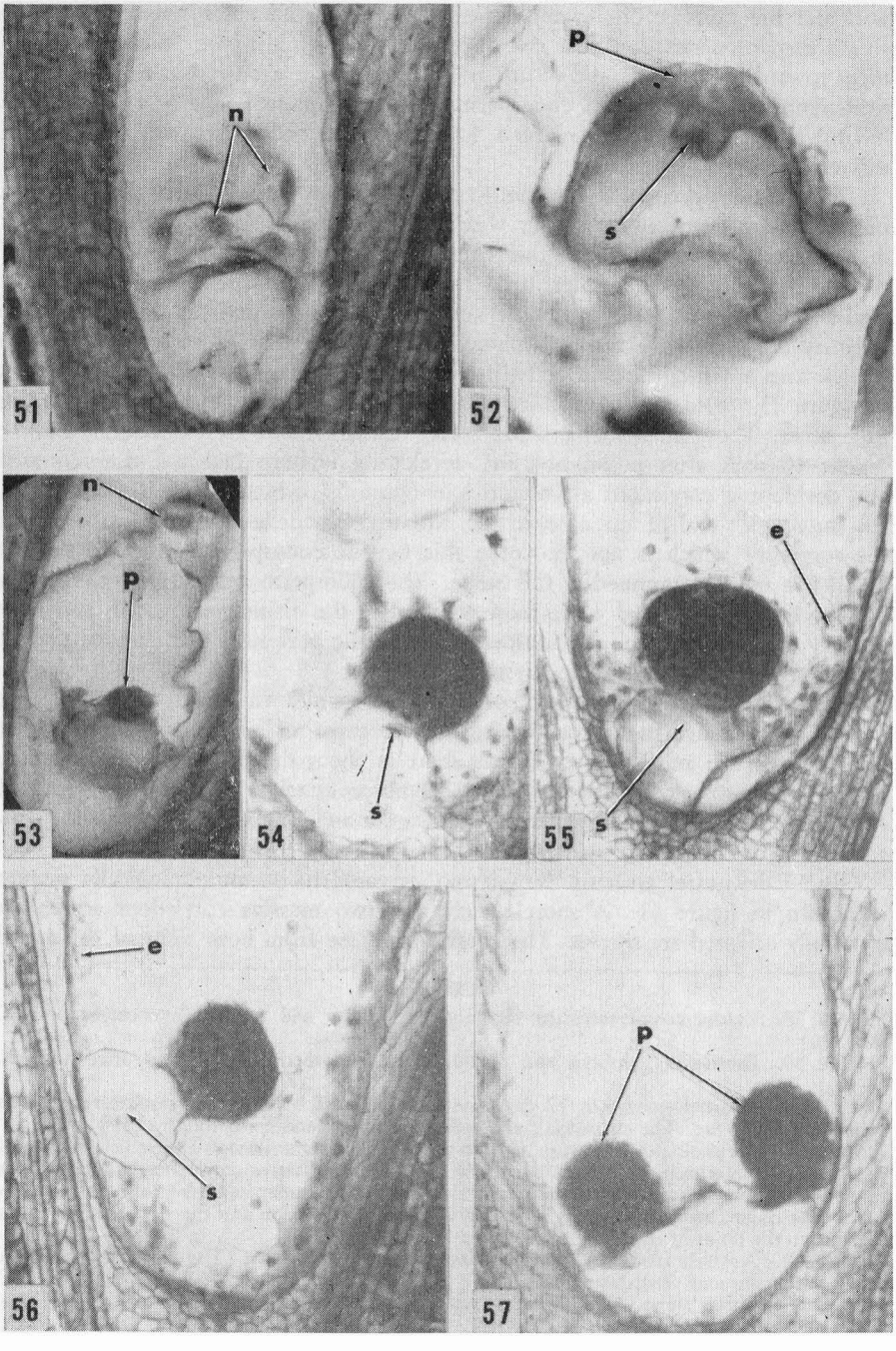

PLATE VIII

wall and the number of developing embryos within an embryo sac. Figure 55 shows suspensor attachment at the micropylar end while figure 56 shows attachment toward the chalazal end of an embryo sac, 923 microns from the micropyle. Both embryo sacs contained endosperm. Figure 57 shows a pair of proembryos having a single but much enlarged suspensor. No endosperm was observed in this embryo sac.

A probable explanation for the occurrence of the twin embryos is the fusion of free nuclei functioning as eggs with each of the two sperm nuclei. The lack of endosperm development in this embryo sac may indicate the malfunction of the polar nuclei when not fertilized by a sperm nucleus. The occurrence of a well-developed twin seedling upon a single hypocotyl-root axis as shown in figure 58, may represent such a twin embryo. The development of embryos from two ovules within a single locule or twin embryo-sacs within a single ovule as shown in figure 23 would result in entirely separate embryos completely separated from each other. These phenomena, while found in the acerola, are exceedingly rare.

At 10 days after pollination the developing embryo had well-differentiated and developing cotyledons as indicated in figure 59, which shows the embryo at the micropylar end of the embryo sac initiating a radicle from cells adjacent to the suspensor which is not shown in this figure. Endosperm cells were present in all five ovules examined at this stage. The endosperm cells nearest the young embryo had disintegrated while those adjacent to the disintegrating cells were not actively dividing. Nuclei of endosperm cells at the periphery and chalazal end of the embryo sac showed active division.

Observations of embryo sacs of immature ovules in which embryos were developing without the accompanying development of endosperm showed that both the embryo and the entire ovule abort at about the 12th day after anthesis. Figure 60 shows a collapsed and aborted embryo attached at the micropylar end of the embryo sac. The aborted embryo is slender and long with clearly distinguishable immature cotyledons and radicle.

By 15 days after anthesis the embryo appeared to be morphologically mature as shown in figure 61. A short radicle and two massive cotyledons somewhat unusually oriented are shown. The cotyledons arose from both sides of the apical

PLATE IX

FIGURE 58. Young acerola seedling showing two shoots and a fused hypocotyl-root axis. (2.31×)

FIGURE 59. Developing embryo and disintegrating endosperm at 10 days after anthesis. (98×)

FIGURE 60. Aborted embryo at 12 days after anthesis. No endosperm was observed in this collapsed embryo sac. The cotyledon *c* and radicle *r* appear slender and long. (159×)

FIGURE 61. Morphologically mature embryo at 15 days after anthesis. The radicle *r* is short and the apical meristem *m* is situated at the base of two massive cotyledons *a* and *b*. One cotyledon *b* shows vascular differentiation and is bent at its center adaxial to the apical meristem. The other cotyledon *a* forms a hood over the first cotyledon and the tip of this hood is adjacent to the point of curvature of the first cotyledon. (14×)

FIGURE 62. A whole fruit and a cross section of a fruit are shown. The cross-sectional view shows the position of the three pyrenes.

FIGURE 63. Three views, dorsal, ventral, and cross section, of the pyrene are shown here. (1.93×)

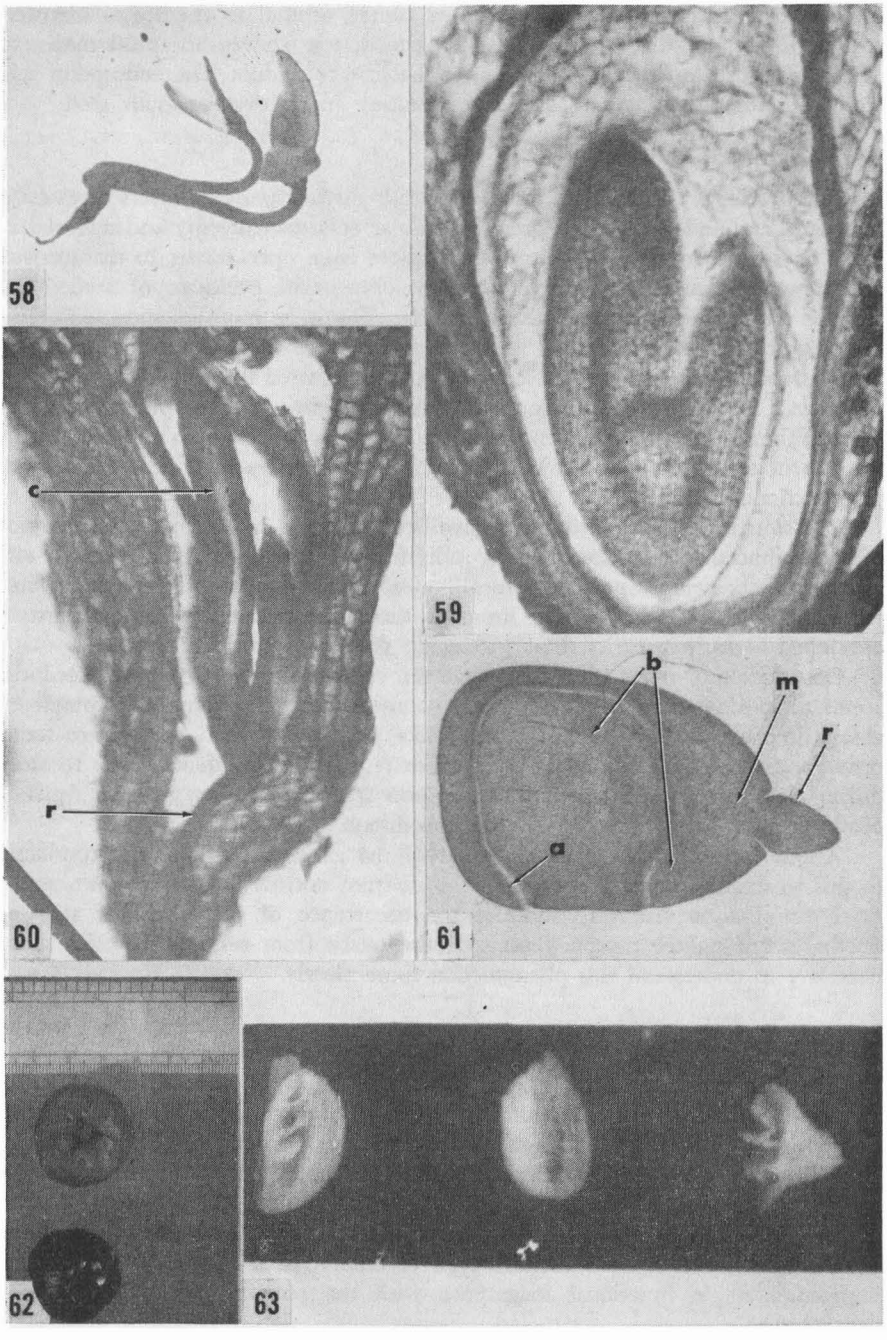

PLATE IX

meristem. One cotyledon was bent at its center, adaxial to the apical meristem so that the tip of this cotyledon rested immediately above the apical meristem. The other cotyledon formed a hood over the first cotyledon. The endosperm cells were completely digested by this time resulting in an exalbuminous seed.

Development of Fruits

The ovaries at anthesis had morphologically distinct pericarp layers of exocarp, mesocarp, and endocarp which grew by cellular enlargement only and matured 21 to 25 days after anthesis. Developmental stages from open flower to mature fruit are shown in figure 1*b*. Growth and development of pericarps of seeded and seedless fruits were morphologically similar. The only morphological difference of fruits at maturity was that at least one of the three stony endocarps (pyrenes) of each seeded fruit contained a seed while none occurred in seedless types. Figure 62 shows a mature whole fruit and a cross section showing three distinct endocarps. The pyrenes in this cross section did not contain any viable seeds and the cavities were shrunken. Figure 63 shows enlargements of pyrenes in dorsal, ventral, and cross-sectional views.

Occasionally, misshapened fruits have been observed in the field. Examination of these abnormally developed fruits indicate the presence of extra carpels with as many as six pyrenes per fruit. In all cases examined none of the pyrenes contained any viable seeds. In most instances these extra carpels were only partially developed at fruit maturity, thus producing grotesquely shaped fruits.

Observation of fruits from more than ten clones and several hundred seedlings seems to indicate that this is a varietal nature, inherent in some and completely absent in most clones and seedlings. While fruits with extra carpels are found throughout the entire fruiting season, an extremely high frequency was recorded during February and March. Fruiting season is normally from around April to November but winter fruiting is not uncommon.

A detailed study of the development of the extra carpels was not conducted in this work since ovaries sectioned were all from normal types. However, superficial examination seems to indicate the occurrence of carpellody of stamens. Further work on the morphogenesis of the ovary from selected varieties seems necessary to understand this phenomenon more clearly.

DISCUSSION

The tetrasporic, 16-nucleate embryo sac in some Malpighiaceae appears to be a specialized type known only from a few genera of scattered families, such as the Compositae, Gunneraceae, Liliaceae, Pandanaceae, Penaeaceae, Piperaceae, and Umbelliferae *(7, 10)*. In this specialized type of embryo-sac development, all four megaspores seem to be functional. Each of the four megaspores gives rise to four nuclei, resulting in a 16-nucleate condition in the mature embryo sac of acerola. This is unlike the usual 8-nucleate type of embryo sacs in which development is from a single functional megaspore with the remaining three megaspores degenerating.

If the monosporic, 8-nucleate embryo sac resulting from the usual three nuclear divisions of the single megaspore is a fundamental type in angiosperms, then the tetrasporic, 16-nucleate embryo sac, resulting from two nuclear divisions of each megaspore, appears to be a modification of the basic type. Thus, this derived type of embryo-sac development has an increase in the number of functional megaspores taking part in the formation of the embryo sac and a decrease in the number of nuclear divisions taking place between the megaspore mother cell and the mature embryo sac.

Results of this study have shown that numerous phenomena causing seedlessness exist in the acerola. In this plant seedlessness may be a result of failure of fertilization but with parthenocarpic fruit development. It may be the result of successful fertilization but abortion of the developing embryo. Embryo abortion in acerola appears to be caused by a failure in endosperm formation. This was clearly indicated in this investigation wherein free nuclear groups were present in embryo sacs containing proembryos but no endosperm cells.

In an embryo sac containing neither endosperm nor free nuclear groups, an aborted embryo with well-differentiated cotyledons was observed 12 days after anthesis (the actual time of embryo abortion, however, was not determined). Further evidences that endosperm is utilized by the developing acerola embryo in cases where endosperm was present, were shown by the digestion of endosperm cells adjacent to the young embryo and the absence of endosperm in the mature seed. As in most angiosperms, endosperm appears to be necessary as nutritional source for the developing acerola embryo. Exceptional cases where endosperm is suppressed and embryo development is often complete are known in Orchidaceae and Podostemonaceae *(18)*.

While ovule development followed "normal" pathways in many instances, many abnormalities were observed. Ovules are normally anatropous at maturity, but abnormal ones were observed to be upside down with their micropyles pointing toward the receptacles or were semi-anatropous with their micropyles pointing toward the attachment of funiculus and placenta. Pollen tube penetration and subsequent fertilization in these abnormally oriented ovules would be hindered. Pollen tubes penetrating the chalaza were not observed in this study, although in *Casuarina suberosa,* this is reported as being the normal situation *(18)*.

Ovule sterility caused by an absence of division or delayed divisions of the megaspore mother cells, disintegration of nucellar cells, and abortion of embryo sacs before anthesis were also observed.

Pollen grains of acerola are generally germinable on the day of anthesis only, and normal germinability is related to anther dehiscence as in most plants. However, pollen germination within the larger lobes of an undehisced anther on the day of anthesis was observed. Germination of pollen grains in undehisced anther sacs in cleistogamous flowers has been reported *(18)* but reports of such occurrences in plants whose flowers are produced well above ground appear rare. Nondehiscence of this particular anther of Clone A at a time when dehiscence of the other anthers of the same flower occurred seems to indicate an abnormal development of the flower.

Preliminary studies on pollen germination and pollen tube growth did not definitely indicate whether or not there was some physiological interference to fertilization (incompatibility) such as slow pollen tube growth or lack of growth. However, reference is made here to an interesting observation in pollen germination and tube growth *in vitro* using stylar extracts. Weakest effects of inhibition were noted when extract and pollen were from the same clone, while strong inhibitory effects were noted when pollen and extract were from different clones. Related to self- and cross-pollinations, the above results would indicate promotion of self-pollination and hinderance of cross-pollination. Yet, pollination studies as reported by Yamane and Nakasone *(17)* show increased fruit set by cross- over self-pollination in all clones studied. Present studies appear inadequate to explain the nature of this discrepancy.

SUMMARY

Various aspects of reproductive morphology of the acerola which included floral differentiation and development, ovule development, gametogenesis, and embryogenesis were studied and summarized in this paper.

Floral bud differentiation from initiation to emergence occurred in 8 to 10 days on primary branches in active vegetative growth. Anthesis was observed approximately 7 days after the date of emergence. Thus, anthesis occurred 15 to 17 days after floral initiation and 21 to 25 days elapsed between anthesis and ripe fruits. Simultaneous production of flowers and fruits on the same plant was a common phenomenon.

There was much variation and abnormality in ovule development, megagametogenesis, and embryo-sac development. What appeared to be the usual or the preponderant type of development in which pollination takes place and seeds are produced is considered here to be the normal development and any deviation in development from this usual type is considered to be abnormal. Both types are described and discussed in this paper. Normal embryo-sac development was like the *Penaea*-type. No nucellar polyembryony was observed in acerola. Parthenocarpy in acerola seemed to be an inherent character and was evidenced by the abnormal types of development.

In general, pollen grains of acerola showed normal development. Two exceptions are described. Pollen grains of acerola were generally germinable only on the day of anthesis and germinability seemed to be influenced by the time of day of anther dehiscence.

Signs of double fertilization within embryo sacs were observed 2 to 3 days after anthesis. The embryo appeared to be morphologically mature by 15 days after anthesis.

Embryo abortion appeared to be caused by failure of endosperm development in some fertilized embryo sacs. Conditions conducive to failure in fertilization were also observed. Development of seedless fruits in spite of these failures indicates parthenocarpy in acerola. Endosperm developed in the normal type, but was completely digested just prior to embryo maturity.

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